双核 Pt(Ⅳ)配合物与 Guanosine-5′-Monophosphate 和 Glutathione 反应的核磁共振光谱研究

范大民　杨晓亮*　郭子建
(南京大学化学化工学院,配位化学国家重点实验室,配位化学研究所,南京　210093)

摘要：合成了一个新型的双核 Pt(Ⅳ)配合物{[*cis*-Pt(NH_3)$_2$Cl(OH)$_2$]$_2$(4,4′-methylenedianiline)}(NO_3)$_2$(化合物 **1**)及相应的 ^{15}N 标记化合物{[*cis*-Pt($^{15}NH_3$)$_2$Cl(OH)$_2$]$_2$(4,4′-methylenedianiline)}(NO_3)$_2$(化合物 ^{15}N-1)。利用 ^{1}H NMR 和 ESMS 进行了结构表征,化合物 ^{15}N-1 的 2D[^{1}H,^{15}N]HSQC NMR 发现,该化合物在水溶液中存在同分异构体。2D[^{1}H,^{15}N]HSQC NMR 技术跟踪了化合物 ^{15}N-1 与 Guanosine-5′-Monophosphate(5′-GMP)和 Glutathione(GSH)的反应。结果显示,5′-GMP 能在 0.5 h 内将化合物 **1** 还原,而 GSH 在 6 h 以后才能够部分的将化合物 **1** 还原。化合物 **1** 所表现出来的反应性能将有利于提高其治疗效果和降低毒副作用。

关键词：抗肿瘤试剂；双核铂(Ⅳ)配合物；DNA；Guanosine-5′-Monophosphate(5′-GMP)；Glutathione(GSH)
中图分类号：O614.82$^+$6；Q525　　**文献标识码**：A　　**文章编号**：1001-4861(2007)07-1206-07

NMR Studies on Reactivity of Dinuclear Platinum(Ⅳ) Complex Towards Guanosine-5′-Monophosphate and Glutathione

FAN Da-Min　YANG Xiao-Liang*　GUO Zi-Jian
(*School of Chemistry and Chemical Engineering, State Key Laboratory of Coordination Chemistry, Coordination Chemistry Institute, Nanjing University, Nanjing* 210093)

Abstract: A dinuclear Pt(Ⅳ) complex {[*cis*-Pt(NH_3)$_2$Cl(OH)$_2$]$_2$(4,4′-methylene-dianiline)}(NO_3)$_2$(compound **1**) was synthesized by the oxidation of {[*cis*-Pt(NH_3)$_2$Cl]$_2$ (4,4′-methylenedianiline)}(NO_3)$_2$, a potent anti-tumor active Pt(Ⅱ) complex. The formation of compound **1** was verified by ^{1}H NMR and ES-MS spectroscopy. Compound **1** shows good water solubility. The ^{15}N-labelled compound **1** (^{15}N-1) was synthesized in order to investigate the reactivity of compound **1** towards guanosine-5′-monophosphate(5′-GMP) and glutathione (GSH) by 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy. It was revealed from the 2D [^{1}H, ^{15}N] HSQC NMR spectra that compound **1** could exist in different isomers in aqueous solution. The reactivity of ^{15}N-1 towards 5′-GMP and GSH was followed by ^{1}H NMR and 2D [^{1}H, ^{15}N] HSQC NMR spectroscopy. The Pt(Ⅳ) isomers of compound **1** can be fully reduced to Pt(Ⅱ) species by 5′-GMP within 0.5 h, while they can only be partly reduced by GSH after 6 h. The reduction products were compared to those of compound **1** and ascorbate incubated for 48 h. The unique reactivity of compound **1** may be desirable for achieving enhanced therapeutic effects and decreased toxic side effects.

Key words: antitumor agent; dinuclear platinum(Ⅱ/Ⅳ) complexes; DNA; guanosine-5′-monophosphate(5′-GMP); glutathione(GSH)

0　Introduction

Since the discovery of cisplatin almost four decades ago, platinum-based drugs have been one of the most widely used anticancer agents worldwide[1~3]. However, due to the side effects such as drug resistance and toxicity[4,5], many platinum complexes have been designed to overcome the drawbacks[6]. Platinum(Ⅳ) complexes

收稿日期:2007-03-28。收修改稿日期:2007-05-25。
国家自然科学基金资助项目(No.20231010)。
*通讯联系人。E-mail:yxlnmr@nju.edu.cn
第一作者:范大民,女,35 岁,博士研究生;研究方向:生物无机化学。

represent a class of promising anti-cancer drugs [7] since they usually show good water solubility and even oral availability[8,9]. Moreover, Pt(Ⅳ) complexes is kinetically inert to substitution reaction compared with their Pt(Ⅱ) counterparts, which can make them more resistance in the gastrointestinal tract, consequently reducing some side effects [10,11]. Furthermore, some platinum (Ⅳ) complexes are active against some tumor cells resistant to cisplatin[12,13].

The most representative Pt(Ⅳ) anti-cancer drug JM216 had been investigated in clinical trial as orally active agent, which stimulated much more interest in the design and synthesis of Pt(Ⅳ) complexes as anti-cancer drugs [14]. For example, trans Pt (Ⅳ) complex JM335 was reported to have greater activity than transplatin and its *cis* analogue [15]. Conjugation of functional axial groups in Pt(Ⅳ) compounds may overcome the resistance or enhance their selectivity to tumor cells[16,17]. More recently, photoreactive Pt(Ⅳ)-diazidodiam (m)ino complexes have been synthesized by Sadler and co-workers demonstrating antitumor potential as photochemtherapeutic agents[18]. However, to the best of our knowledge, there are very few reports about di-nuclear platinum(Ⅳ) complexes. In this study, a dinuclear Pt(Ⅳ) complex {[*cis*-$Pt(NH_3)_2Cl(OH)_2]_2$(4,4′-methylenedianiline)}$(NO_3)_2$ (compound **1**) was synthesized by the oxidization of {[*cis*-$Pt(NH_3)_2Cl]_2$ (4,4′-methylenedianiline)}$(NO_3)_2$, a potent anti-tumor active Pt(Ⅱ) complex reported recently by our group[19]. Its reactivity towards guanosine-5′-monophosphate (5′-GMP) and glutathione (GSH) was investigated by the ^1H NMR and 2D [^1H, ^{15}N] HSQC NMR spectroscopy.

1 Experimental

1.1 Reagents

Cis-[$Pt(NH_3)_2Cl_2$] and 4,4′-methylenedianiline (L) were purchased from Sigma-Aldrich; glutathione (GSH), disodium salt of guanosine-5′-monophosphate (5′-GMP) and ascorbic acid were from Sigma. Cis-[Pt($^{15}NH_3)_2Cl_2$] was prepared according to the published method using $(^{15}NH_4)_2SO_4$ as starting material[20]. Common reagents used in the experiment were all of analytical grade.

1.2 Analytical methods

The ^1H NMR spectra were recorded in D_2O on a Bruker DRX 500 MHz NMR spectrometer at 298 K, using TMS as an external reference (δ=0 ppm). The 2D [^1H, ^{15}N] HSQC NMR spectra were recorded on a Bruker DRX 500 MHz NMR spectrometer in 10% D_2O/90% H_2O. Water suppression was achieved by using gradient pulses. The mass spectra were recorded on an LCQ electron spray mass spectrometer (ES-MS, Finnigan). The pH value of the solutions were adjusted by diluted NaOH solution and measured on a PHS-3C pH meter equipped with a Phonix Ag-AgCl reference electrode calibrated with standard pH buffer solutions.

1.3 Synthesis

{[*cis*-$Pt(NH_3)_2Cl(OH)_2]_2$(4,4′-methylenedianiline)}$(NO_3)_2$ (compound **1**) {[*cis*-$Pt(NH_3)_2Cl]_2$(4,4′-methylenedianiline)}$(NO_3)_2$ (100 mg, 0.12 mmol) synthesized as described[19] was dissolved in H_2O (50 mL), then 30 % H_2O_2 (15 mL) was added. The reaction mixture was stirred for 1 h at 70 ℃ in the darkness, after which the stirring was continued for 24 h at room temperature in the darkness. The final product was filtered and the filtrate was evaporated to dryness under reduced pressure at 35 ℃, then H_2O (30 mL) was added and filtered again. The volume of the filtrate was reduced to 5 mL under reduced pressure. A yellow precipitate was produced by adding acetone (60 mL) to the filtrate, then the precipitate was collected by centrifuging. The solid was washed repeatedly with a small amount of ethanol, acetone, diethyl ether respectively and dried in vacuum. Yield: 40mg (36%). ^1H NMR (D_2O) δ(ppm): 7.61 (s, 8 H), 4.24 (s, 2 H).

{[*cis*-$Pt(^{15}NH_3)_2Cl(OH)_2]_2$(4,4′-methylenedianiline)}$(NO_3)_2$. (compound ^{15}N-1) was synthesized according the same method as described above for compound **1** using ^{15}N-labeled {*cis*-$[Pt(^{15}NH_3)_2Cl]_2$(4,4′-methylenedianiline)}$(NO_3)_2$ as starting material.

1.4 Interactions with biomolecules

The reaction of compound **1** with 5′-GMP at 1:2 molar ratio in 0.5 mL 10% D_2O/90% H_2O (pH 7.4) was followed by 2D [^1H, ^{15}N] HSQC NMR spectra. Compound ^{15}N-1 was used for 2D [^1H, ^{15}N] HSQC NMR studies.

The reaction of compound ^{15}N-1 with GSH at 1:4 ratio in 0.5 mL 10% D_2O/90% H_2O (pH value of 2.97) was followed by ^{1}H NMR spectra and 2D [^{1}H, ^{15}N] HSQC NMR spectra.

2 Results and discussion

2.1 Characterization of compound 1 and compound ^{15}N-1

Compound **1** shows good water solubility, therefore the following studies on its reactivity toward guanosine-5-monophosphate (5′-GMP) and glutathione (GSH) were performed in aqueous solution. The formation and purity of compound **1** was confirmed by ^{1}H NMR and ESMS. In the ^{1}H NMR spectrum (Fig.1A), both methylene and phenyl signals (4.24 ppm, -CH_2; 7.61 ppm, -Ph-H) shifted significantly to the lower field compared to those of the starting material dinuclear platinum (Ⅱ) complex [19]. Since only one set of signals were observed, both amine groups of L must bind to Pt (Ⅳ) equivalently. In the ES-MS spectrum of **1** (Fig.1B), a major peak at *m/z* 396.8 was observed, which could be attributed to the two positively charged species $\{[Pt(NH_3)_2Cl(OH)_2]_2L\}^{2+}$ ($Pt_2C_{13}H_{30}Cl_2N_8O_{10}$). The isotopic distribution pattern of this peak matches perfectly with the simulated one. These data suggest the formation of compound **1**.

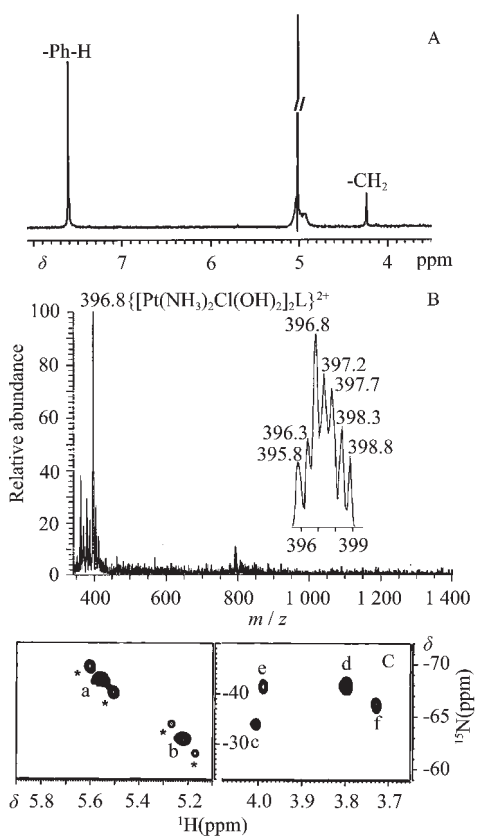

Fig.1 ^{1}H NMR (A, D_2O, 298 K) and ES-MS (B, methanol, positive mode) spectra of compound **1** and the 2D [^{1}H,^{15}N] HSQC NMR spectrum (C, 10% D_2O/90% H_2O, 298 K) of ^{15}N-compound **1**. (Note: the crosspeaks c, d, e, f were magnified in order to be clearly seen)

The 2D [^{1}H, ^{15}N] HSQC NMR spectrum of ^{15}N-1 is shown in Fig.1C. The two cross-peaks a (5.51/−42.96 ppm) and b (5.16/−31.30 ppm) appeared in $^{15}NH_3$-Pt (Ⅳ) region (* representing Pt (Ⅳ) satellite peaks) [21]. These two peaks could be assigned to $^{15}NH_3$Pt (Ⅳ) Cl group and $^{15}NH_3$Pt (Ⅳ) amine of L, respectively [21]. Cross-peaks c (4.01/−64.33 ppm), d (3.79/−68.76 ppm), e (3.98/−67.96 ppm) and f (3.73/−65.93 ppm) were also observed which are located in the chemical shift region assignable to $^{15}NH_3$-Pt (Ⅱ), suggesting that some Pt(Ⅱ) species be present in compound **1**. For example, cross-peaks c and d can be assigned to un-reacted starting material [19]. According to the integral proportion of the cross-peaks, the Pt (Ⅳ) species accounts for ca 85% in the whole system. It was noted that the intensity of cross-peak a is higher than that of cross-peak b, suggesting that other Pt (Ⅳ) species such as trans-isomer may exist in the system.

In order to confirm the assignment of these cross-peaks, compound **1** was reduced by ascorbate. The reason for choosing ascorbate as reductant is that ascorbate would not react continuously with the corresponding Pt(Ⅱ) species[22], thus the complication of the assignment could be avoided. The ^{15}N-1 was incubated with sodium ascorbic at 1:10 ratio in 10% D_2O/90% H_2O at 37 ℃ for 48 h in the darkness. Before incubation, the pH value of the mixture of Pt(Ⅳ) species and ascorbic acid was adjusted to 7.4 with NaOH solution using pH meter. From the 2D [^{1}H, ^{15}N] HSQC NMR (Electronic supplemental material is available from the corresponding author for the interested readers upon request.), it can be observed that the cross-peaks assigned to Pt(Ⅳ) species are all disappeared, suggesting compound **1** has been fully reduced to Pt (Ⅱ). Four

cross-peaks, R_1 to R_4, appear in the $^{15}NH_3$-Pt(Ⅱ) region. The location of cross-peaks R_1 (4.01/−64.33 ppm) and R_2 (3.79/−68.76 ppm) are consistent with that of the starting Pt(II) complex A, thus they could be assigned to $^{15}NH_3$ trans to ligand and $^{15}NH_3$ trans to chloride group in A. The location of cross-peaks R_3 and R_4 are consistent with that of cross-peaks e and f in the spectra of ^{15}N-1. The existence of cross-peaks R_3 and R_4 suggests that when compound **1** be fully reduced to its Pt(Ⅱ) species by ascorbate, the starting material A is not the only product. These data suggest that cross-peaks R_3 and R_4 may be derived from the isomers of A (B or C in Fig.2). From the integral proportion of the cross-peaks in the 2D [^{1}H, ^{15}N] HSQC NMR spectrum, the possibility on the existence of isomer C is very small. Thus the cross-peaks R_3 and R_4 could be respectively assigned to $^{15}NH_3$ trans to $^{15}NH_3$ and $^{15}NH_3$ trans to amine of L in complex B, and the chemical shift of $^{15}NH_3$ trans to chloride group in complex B is overlapped with that of complex A as shown from the integration. According the assignment of resulting Pt(Ⅱ) species, the cross-peaks for compound **1** can also made and they correspond to the two Pt(Ⅳ) isomers (Fig.3, **A1** and **B1**). Therefore, the final assignment of the cross-peaks in Fig.1C is listed in Table 1. The cross-peak a could be assigned to $^{15}NH_3$-Pt(Ⅳ)-Cl group in complex **A1** and **B1**, moreover, the resonance of $^{15}NH_3$-Pt(Ⅳ)-$^{15}NH_3$ in complex **B1** are overlapped with that of $^{15}NH_3$-Pt(Ⅳ)-Cl group in complex **A1** and **B1**. The cross-peak b could be assigned to $^{15}NH_3$-Pt(Ⅳ)-amine of L in complex **A1** and **B1**.

When starting Pt(Ⅱ) compound **A** was oxidized, it was partially transformed into its isomer complex B to produce corresponding complex **A1**. The heating process during the oxidization may be the reason to cause such an isomerization.

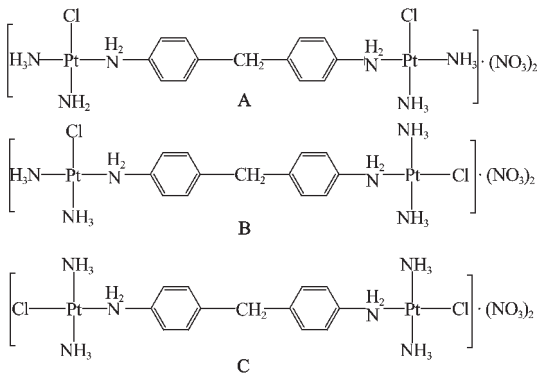

Fig.2　Isomers of the starting Pt(Ⅱ) compound A

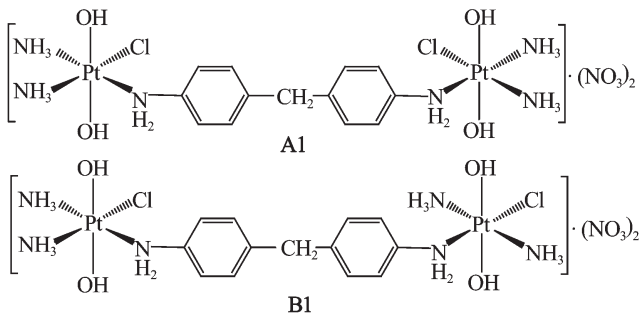

Fig.3　Dinuclear platinum(Ⅳ) complex **A1** and its isomer **B1**

Table 1　Assignment of cross-peaks for ^{15}N-1 and the assignment of cross-peaks (R1, R2, R3, R4) resulted from the reduction of ^{15}N-1 by ascorbate in the 2D [^{1}H, ^{15}N] HSQC NMR spectra.

Cross-peak	δ (^{1}H / ^{15}N) / ppm	Assignments
a	5.51 / −42.96	$^{15}NH_3$-Pt(Ⅳ)-Cl in complex A1 and **B1**, $^{15}NH_3$ − Pt(Ⅳ) − $^{15}NH_3$ in complex **B1**
b	5.16 / −31.30	$^{15}NH_3$-Pt(Ⅳ)-amine of L in complex **A1** and **B1**
c(R_1)	4.01 / −64.33	$^{15}NH_3$ *trans* NH_2-L in complex **A**
d(R_2)	3.79 / −68.76	$^{15}NH_3$ *trans* Cl^- in complex **A** and complex **B**
e(R_3)	3.98 / −67.96	$^{15}NH_3$ *trans* $^{15}NH_3$ in complex **B**
f(R_4)	3.73 / −65.93	$^{15}NH_3$ *trans* NH_2-L in complex **B**

2.2　Interaction of 1 with GMP

Since DNA is the potential target of Pt(Ⅳ) complexes, the reaction of ^{15}N-1 with 5′-GMP (Fig.4) at 1:2 molar ratio was followed by 2D [^{1}H, ^{15}N] HSQC NMR spectra for 24 h (Fig.5). A series of new cross-peaks were observed during the reaction. The assignments of these cross-peaks are summarized in Table 2.

Pt(Ⅳ) species completely disappear after 0.5 h and the cross-peaks corresponding to Pt(Ⅱ) species are observed. Moreover, the spectrum at 0.5 h is in agreement with that of the reduction of Pt(Ⅳ) species by ascorbate, suggesting that Pt(Ⅳ) species be completely

reduced to the corresponding Pt(Ⅱ) species by 5′-GMP. The four cross-peaks a (3.79/−68.76 ppm), b (4.01/−64.33 ppm), c (3.98/−67.96 ppm) and d (3.73/−65.93 ppm) at 0.5 h could be assigned to corresponding complex **A** and **B**. The reaction procedure after 0.5 h was similar to that observed for the starting Pt(Ⅱ) compound A with 5-GMP[19]. Cross-peaks such as e, f, g, h, i could be assigned to the Pt(Ⅱ)-adducts of 5′-GMP (or oxo-GMP). The cross-peaks corresponding to Pt(Ⅱ) complexes **A** and **B** fully disappeared at 24 h, suggesting the completion of the reaction.

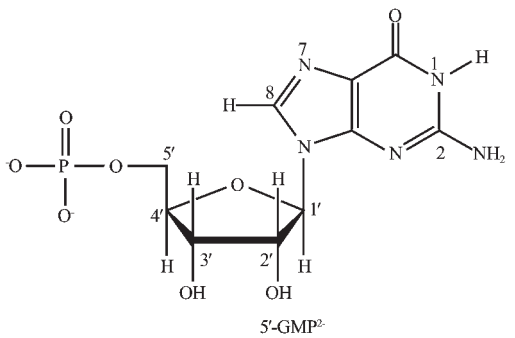

Fig.4 Structure of guanosine-5′-monophosphate ($5'$-GMP^{2-})

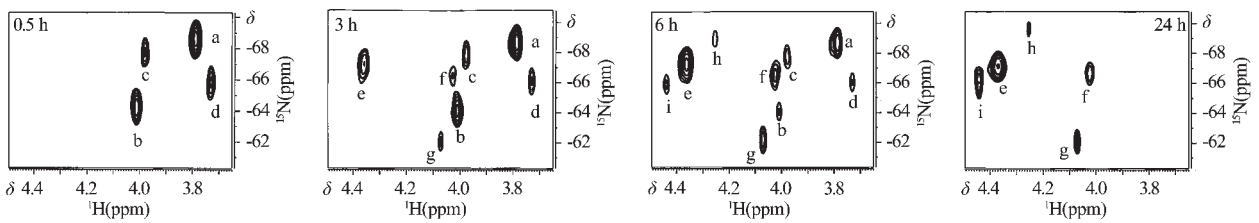

Fig.5 Selected 2D [^{1}H, ^{15}N] HSQC NMR spectra for the reaction of ^{15}N-compound **1** with 5′-GMP at 1:2 molar ratio during 24 h

Table 2 Assignment of cross-peaks observed in the 2D [^{1}H, ^{15}N] HSQC NMR spectra for the reaction of ^{15}N-compound 1 with 5′-GMP

Cross-peak	δ (^{1}H / ^{15}N) ppm	Assignment
a	3.79 / −68.76	$^{15}NH_3$ *trans* Cl^- in complex **A** and complex B
b	4.01 / −64.33	$^{15}NH_3$ *trans* NH_2-L in complex **A**
c	3.98 / −67.96	$^{15}NH_3$ *trans* $^{15}NH_3$ in complex **B**
d	3.73 / −65.93	$^{15}NH_3$ *trans* NH_2-L in complex **B**
e	4.36 / −67.15	$^{15}NH_3$ *trans* GMP in **A** or **B**-GMP (oxo-GMP) adduct
f	4.03 / −66.50	$^{15}NH_3$ *trans* NH_2-L in **A** or **B**-GMP (oxo-GMP) adduct
g	4.07 / −62.17	$^{15}NH_3$ *trans* $^{15}NH_3$ in **B**-GMP (oxo-GMP) adduct, $^{15}NH_3$ *trans* NH_2-L in mono-**A**-GMP (oxo-GMP)adduct
h	4.25 / −68.95	$^{15}NH_3$ *trans* Cl^- in mono-**A**-GMP (oxo-GMP) adduct
i	4.44 / −66.01	$^{15}NH_3$ *trans* GMP isomer of **A**-GMP (oxo-GMP) adduct

2.3 Interaction with GSH

Because GSH is one of the most important reductant in biomolecules, the interaction of ^{15}N-1 with GSH at 1:4 molar ratio was followed by ^{1}H NMR and 2D [^{1}H,^{15}N] HSQC NMR spectroscopy for 24 h to investigate if GSH can also reduce compound **1**. A series of new cross-peaks were observed during the reaction from 2D [^{1}H, ^{15}N] HSQC NMR spectra (Fig.6). The assignments of these cross-peaks are summarized in Table 3.

Both the intensity of two cross-peaks a and b due to Pt(Ⅳ) species and the intensity of cross-peaks c, d, e and f due to Pt(Ⅱ) species show no significant decrease after 0.5 h, suggesting that compound **1** could not be reduced readily to Pt(Ⅱ) species by GSH. It is interesting to note that a new cross-peak g appears at 7.0/0.67 ppm, which could be attributed to the released $^{15}NH_3$ ($^{15}NH_4^+$) from Pt(Ⅱ) species due to the trans effect of S-GS when GSH coordinated with the Pt(Ⅱ) center. However, the cross-peak corresponding to $^{15}NH_3$ trans to S-GS is not observed at 0.5 h. Two new cross-peaks h and i are observed after 3 h. The former could be assigned to $^{15}NH_3$ trans to S-GS and the latter to $^{15}NH_3$ trans to the amine of L in the substituted

end of mono-GS adduct. The line shape of the cross-peaks c and d change slightly, suggesting that the chemical shifts of the two $^{15}NH_3$ groups in un-substituted end of mono-GS adduct be partly overlapped with that of the cross-peaks c and d. Three new cross-peaks j, k and l appear in Pt(Ⅳ) region after 6 h which should be resulted from the substitution reaction of the axial hydroxyls of Pt(Ⅳ) species by GSH. A new cross-peak m appears in Pt(Ⅱ) region could be assigned to $^{15}NH_3$ trans to S-GS. It is likely that the Pt(Ⅳ)-GS adduct is reduced to Pt(Ⅱ) species which continues to react with GSH to form Pt(Ⅱ)-GS adducts. In order to solve the ambiguity, ^1H NMR spectra of the same reaction were recorded (Electronic supplemental material is available from the corresponding author for the interested readers upon request). The intensity of the peak due to Pt(Ⅳ) species apparently decreases compared to that at 3 h. Two new peaks appeared at 7.0 ppm and 6.9 ppm, which could be assigned to the corresponding Pt(Ⅱ) species and Pt(Ⅱ)-GS adducts. In the meantime, the peak at 3.25 ppm assignable to the CH_2-S of GSH partly shifts upfield, suggesting that the partial Pt(Ⅳ) reduction be accompanied by the partial oxidation of GSH to GSSG. At 24 h, the intensity of cross-peaks a and b in the 2D [^1H, ^{15}N] HSQC NMR spectra apparently decrease. In corresponding ^1H NMR spectra, it can be observed that the decrease of the peak due to Pt(Ⅳ) species in intensity is concomitant with the increase of Pt(Ⅱ) species and Pt(Ⅱ)-GS adducts in intensity. The cross-peak g increases ever

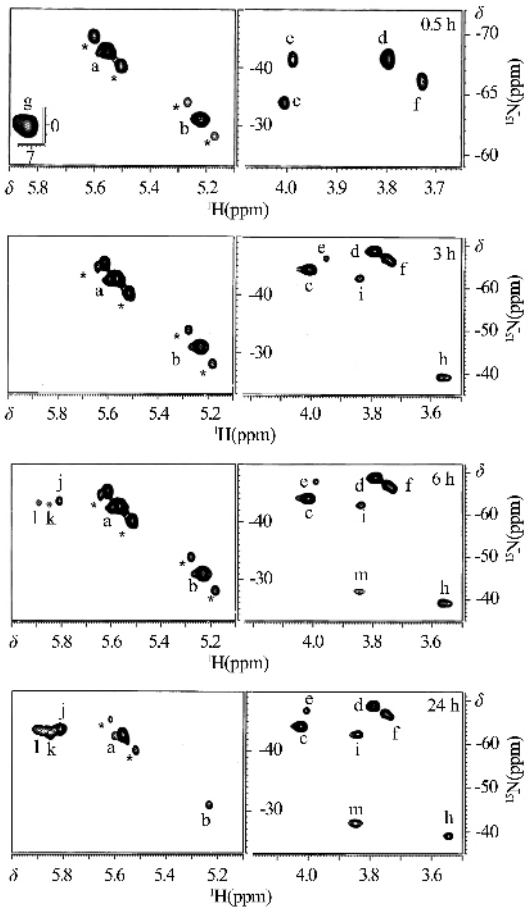

Fig.6　Selected 2D [^1H, ^{15}N] HSQC NMR spectra for the reaction of ^{15}N-compound **1** with GSH at 1:4 ratio during 24 h. (Note: the crosspeaks c, d, e, f g, h, i, m were magnified in order to be clearly seen)

Table 3　Assignment of cross-peaks observed in the 2D [^1H, ^{15}N] HSQC NMR spectra for ^{15}N-1 with GSH

Cross-peak	δ(^1H / ^{15}N) / ppm	Assignments
a	5.51 / −42.96	$^{15}NH_3$ -Pt(Ⅳ) -Cl^- in complex **A1** and **B1**, $^{15}NH_3$ -Pt(Ⅳ) $^{15}NH_3$ in complex **B1**
b	5.16 / −31.30	$^{15}NH_3$ -Pt(Ⅳ) -amine of L in complex **A1** and **B1**
c	4.01 / −64.33	$^{15}NH_3$ *trans* NH_2-L in complex **A**
d	3.79 / −68.76	$^{15}NH_3$ *trans* Cl^- in complex A and complex **B**
e	3.98 / −67.96	$^{15}NH_3$ *trans* $^{15}NH_3$ in complex **B**
f	3.73 / −65.93	$^{15}NH_3$ *trans* NH_2-L in complex **B**
g	7.0 / −0.67	$^{15}NH_3$ ($^{15}NH4+$) from Pt(Ⅱ) species due to the *trans* effect of S-GS
h	3.57 / −39.17	$^{15}NH_3$ *trans* S-GS in mono-A-GS adduct
i	3.84 / −62.68	$^{15}NH_3$ *trans* NH_2-L in substituted end of mono-A-GS adduct
j	5.81 / −43.77	Pt(Ⅳ)-GS adduct
k	5.85 / −43.15	Pt(Ⅳ)-GS adduct
l	5.89 / −43.30	Pt(Ⅳ)-GS adduct
m	3.84 / −39.22	$^{15}NH_3$ *trans* to S-GS

since its appearance during 24 h as shown from [^{1}H, ^{15}N] HSQC NMR spectra.

3 Conclusions

This work demonstrated that the unique reactivity of the platinum(Ⅳ) complex {[*cis*-Pt $(NH_3)_2Cl(OH)_2]_2$ (4,4′-methylenedianiline)}$(NO_3)_2$ (**1**) towards 5′-GMP and GSH. It is quite remarkable that compound (**1**) can be fully reduced to Pt(Ⅱ) species by 5′-GMP within 0.5 h. Such a fast reaction has never been observed previously for Pt(Ⅳ) compounds, which could be helpful for the further rational design of Pt(Ⅳ)-based antitumor complexes. The inertness of compound (**1**) towards GSH may be another interesting feature required for the design of platinum-based anti-tumor agents with reduced side effects.

References:

[1] Boulikas T, Vougiouka M. *Oncol. Rep*., **2003**,**10**:1663~1682

[2] Pasini A, Zunino F, Angew. *Chem. Int. Ed. Engl*., **1987**,**26**:615~624

[3] Fuertes M A, Castilla J, Perez J M, et al. *Anti-Cancer Agents*, **2002**,**2**:539~551

[4] Kelland L R. *Drugs*, **2000**,**59**:1~8

[5] Dempek W, Voigt W, Grothey A, et al. *Anti-Cancer Drugs*, **2000**,**11**:225~236

[6] Galanski M, Jakupec M A, Keppler B K Curr. *Med. Chem.*, **2005**,**12**:2075~2094

[7] Mellish K J, Kelland L R, Harrap K R *Br. J. Cancer*, **1993**, **68**:240~250

[8] Bramwell V H C, Crowther D, OMalley S, et al. *Cancer Treat. Rep*., **1985**,**69**:409~416

[9] Rahman A, Wolpert-DeFilippes M K, Goldin A, et al. *Cancer Res*., **1988**,**48**:1745~1752

[10]Ellis L A, Er H M, Hambley T W. *Aust. J. Chem*., **1995**,**48**:793~806

[11]Kelland L R, Murrer B A, Abel G, et al. *Cancer Res*., **1992**, **52**:822~828

[12]Khokhar A R, Deng Y, Kido Y. *J. Inorg. Biochem*., **1993**,**50**:79~87

[13]Kido Y, Khokhar A R, Siddik A H. *Biochem. Pharmacol*., **1994**,**47**:1635~1642

[14]Wong E, Giandomenico C M. *Chem. Rev*., **1999**,**99**:2451~2466

[15]Lemma K, Shi T, Elding L. *Inorg. Chem*., **2000**,**39**:1728~1734

[16]Wee H A, Sébastien P, Dyson P J, et al. *J. Med. Chem*., **2005**,**48**:8060~8069

[17]Barnes K R, Kutikov A, Lippard S J. *Chem. Biol*., **2004**,**11**:557~564

[18]Mackay F S, Woods J A, Sadler P J, et al. *Chem. Eur. J*., **2006**,**12**:3155~3161

[19]Fan D M, Yang X L, Guo Z J, et al. *J. Biol. Inorg. Chem*., **2007**,**12**:DOI10.1007/s00775-007-0214-1

[20]Dhara S. *Indian. J. Chem*., **1970**,**8**:193

[21]Berners-Price S J, Ronconi L, Sadler P J. *Prog. Nucl. Mag. Res*., **2006**,**Sp49**:65~98

[22]Hall M D, Hambley T W. *Coord. Chem. Rev*., **2002**,**232**:49~67